REMARKS

Objections to the Specification:

Applicants have addressed the objections to the Specification as detailed in the Office Action of April 27, 2009 by providing the Abstract, headings and correcting errors as provided herein. With regard to the objection to the specification for allegedly failing to include sequence identifiers, Applicants respectfully submit that the instant specification was amended to include said sequence identifiers by means of a Preliminary Amendment filed on July 10, 2006. Although the Examiner has requested that the sequences recited on pages 4-5 of the specification be deleted, Applicants believe that it is not improper for the Applicant to include such sequence information in the body of the application, and request further clarification as to this objection.

Incorporation by Reference:

As detailed on Page 5 and 6 of the outstanding Office Action, the Examiner has alleged that the Applicants have improperly attempted to incorporate essential subject matter by reference, specifically with regard to markers SG129 and SG34.

In response, Applicants submit that the prior art cited in the Office Action, *i.e.*, Primard-Brisset et al., 2005, Theoretical and Applied Genetics 111:736-746, reports the corresponding nucleotide sequences. Thus, the disclosure of the two primers, "SG129" and "SG34" strictly in terms of these abbreviations and without the corresponding nucleotide sequences, is in full compliance with the requirements under Section 112, first paragraph (written description). In *Falko-Funter Falkner v. Inglis*, 448 F.3d 1357 (Fed. Cir. 2006), the Federal Circuit ruled that the written description requirement does not require recitation of *known* genes or sequences. Its reasoning was as follows:

"Falkner argues, *inter alia*, that the Inglis specifications do not adequately describe the poxvirus invention, in light of *Eli Lilly*, becaue they do not describe the 'essential regions' of any poxvirus. 119 F.3d 1559. We note, in addition, that Inglis did not attempt to incorporate by reference any literature that described the DNA sequence of the poxvirus genome and the locations of the 'essential regions.' However, it is the binding precedent of this court that Eli *Lilly* does <u>not</u> set forth a <u>*per se*</u> rule that whenever a claim limitation is directed to a macromolecular sequence, the specification must always recite the gene or sequence, regardless of whether it is known in the prior art. . . Indeed, the forced recitation of known sequences in patent disclosures would only add unnecessary bulk to the specification. Accordingly, we hold that where, as in this case, accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences (here 'essential genes'), ***satisfaction of the written description requirement does not require the recitation or incorporation by reference [footnote omitted](where permitted) of such genes and sequences.***"

Falko-Funter Falkner, 448 F.3d at 1367-68 (emphasis added).

This case followed the Federal Circuit's prior ruling in *Capon v. Eshhar,* 418 F.3d 1349, 1356 (Fed Cir. 2005) (ruling that chimeric genes prepared from known DNA sequences of known function need not be analyzed and reported in the specification, and that there is no requirement to provide a re-description of what was already known.)

In view of the foregoing, Applicants submit that the disclosure in question is not "essential" so as to invoke the

requirements regarding incorporation by reference. Reconsideration and withdrawal of the objection are respectfully requested.

Indefiniteness:

In response to the Examiner's rejection of claims 37-47 under 35 USC 112 for indefiniteness as detailed on Pages 6 and 7 of the outstanding Office Action, Applicants believe that the amendments to the claims provided herein obviate these rejections, and reconsideration and withdrawal of these rejections is respectfully requested.

Enablement:

Claim 41 is currently rejected under 35 USC 112 as allegedly failing to comply with the enablement requirement. In response, Applicants respectfully direct the Examiner's attention to arguments provided above in response to the Examiner's allegations of improper incorporation by reference.

Plant Deposit:

In response to the rejection of Claim 45 for lack of enablement as detailed on Page 8 of the instant Action, Applicants have amended this claim herein to include the deposit information relevant to the claimed subject matter. Support for this amendment can be found, for example, in the patent application document EP 1 493 328 published on February 10, 2005 (claim 10), which corresponds to the publication of the second priority document claimed for the present application (EP application filed on December 8, 2003 under the number EP 03293057.0). Applicants respectfully submit that National Collections of Industrial, Food and Marine Bacteria (NCIMB), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland, United Kingdom, is a recognized Depositary Authority under the Budapest Treaty.

Starting Material:

Claims 37-44 and 46-47 are currently rejected under 35 USC 112, first paragraph for alleged lack of enablement as detailed on Pages 9-12 of the outstanding Office Action.

In response, Applicants respectfully submit the following arguments:

Breeding restorer lines for the Ogu-INRA Cytoplasmic Male Sterility (cms) system in rapeseed (Brassica napus L.) has been a major objective during the past years in order to develop rapeseed hybrids. Extensive backcross and pedigree breeding was necessary to improve their female fertility and to get restorer lines with a low glucosinolate content (low GC) (Delourme et al. 1991; 1995) from the starting Ogura rapeseed restored material obtained by Heyn (1976). This breeding was difficult because recombination around the introgressed radish genome fragment in the B. napus restorer line is very reduced (Delourme et al. 1994, 1998). Breeding resulted in restorer lines that were used for F1 hybrid breeding (Delourme et al., 1999) but these restorer lines still encountered some difficulties (introgression rearrangements, possible linkage with negative traits) due to the large size of the radish introgression.

The introgression proved to be sometimes unstable, losing partial segments. The development of low GC restorer lines often occurred through deletion of a part of the radish introgression (Delourme et al. 1992; 1995; 1999). But these events did not result from recombination with the homologous chromosome of rapeseed as losses of radish information were not necessarily compensated by the recovery of the corresponding rapeseed information. Then, some double low restorer lines were obtained with a shorter radish introgression but, since they had not recovered all the rapeseed genetic information, they showed a very poor agronomic value. That was the case of 'R211' line but other

lines of the same type were also obtained (such as in Delourme et al. 1992; 1999). Then, recombination and recovery of rapeseed information through classical breeding was thought to be very difficult and would need the screening of large numbers of plants.

A way to get improved restorer lines was to attempt to force recombination between the radish insertion and the homologous B. napus chromosome. For this the idea was to perform irradiation on an heterozygous restored rapeseed plant, carrying the Rfo introgressed radish segment (with the shortest segment as possible) associated to a normal rapeseed chromosome, in order to generate chromosomal lesions and, during meiosis, natural repair mechanisms and thus induce recombination between the homologous chromosome segments, transmissible through pollen grains.

Applicants' objective was to get an improved low GC restorer B. napus line, so the program was initiated from a low GC spring restorer selected line (named 'R211') which carries a deleted radish insertion e.g. having lost the radish Pgi-2 allele (as in Delourme and Eber 1992; 1995; 1999). This type of 'R211' line was also chosen because it showed a very poor agronomic value. Among its most deleterious characters observed, mainly three traits were very affected: (i) the fertile ratio in F2 progenies derived from this material is lower than expected (Delourme et al. 1995), (ii) its low female fertility is expressed by a poor seed set in selfing, (iii) the whole homozygous plant exhibited a very poor vigour.

Then, selection in F2 generation derived from irradiated material could be based on phenotypic observations of these three chosen traits, which can be easily assessed on a high number of plants.

Process for obtention of double low cms line of Brassica napus comprising a deleted radish insertion and having a poor agronomic value, as "R211" type is well known by the skill person

(see Document A of the Appendix (attached herein), specifically, the "Introduction" and Materials and Methods part, see also the cited references in Document A; see also Document B also provided herein as part of the Appendix, "Introduction" part where it is indicated that different low-glucosinolate restorer lines presenting radish introgression and having lost the radish Pgi-2allele have been already described and characterized (see the end of the "Introduction" part and "Material and Methods" part).

For example, in view of the publications Pellan-Delourme et al., 1988 (Genome, 30:234-238; and Delourme et al., In proceeding of the 9th International Rapeseed Congress, 4 to 7 July 1995, Cambridge (GB), vol. 1 to 4; A3 (pages 6-8) see copy enclosed), double low restorer lines having poor agronomic value and low glucosinolate content can be easy obtained using visual screening and glucosin.

One family, 'R2000 family' showing the expected positive expression of the analysed characters was selected and characterised by molecular markers defined around the restorer gene. This 'R2000' family has a new combination of these markers indicating that a recombination event took place in the irradiated heterozygous plant since genetic information carried by the homologous rapeseed chromosome (B. oleracea genome) was found to be linked to the radish introgression in 'R2000'.

Thus, Applicants have demonstrated that a specific combination of 5 markers is present in the double low restorer line of Brassica napus line having agronomic value which is desired to obtain, and that these five markers can be used in a method of producing such Brassica napus line for the step of selection, renders this step of selection more easy to implement after the step of irradiating.

As such, Applicants respectfully submit that the specification provides sufficient guidance such that one of skill in the art would be able to make and use the claimed invention.

Concerning the predictability, or reproducibility, of the production of the Bassica napus plants of the present invention, the Applicants have selected one family out of 1183 that were screened in the field. To obtain such event with this frequency, at a probability of 99%, 5450 families should be screened, which is not at all incompatible with current numbers of plants observed by plant breeders. Indeed, with the use of the 5 markers we have developed, this work can be done more easily since the screening of a recombination event can be performed using these PCR markers and the agronomic value of the screened plants can be checked afterwards.

Anticipation:

Claims 39, 44 and 47 are currently rejected under 35 USC 102(b) in view of Delourme et al., 1992, *Theoretical and Applied Genetics* 85:222-228 and Delourme et al., 1999 *10th Rapeseed Conference, Canaberra*, Pages 26-29 as detailed on Pages 12-14 of the outstanding Office Action.

In response, Applicants reiterate the arguments presented hereinabove, and particularly with regard to the characterization of the cited art. Applicants further acknowledge the Examiner's conclusion that Claims 37-38, 40-43 and 45-46 are deemed free of the prior art. As claim 39 is dependent from claim 37, claim 44 is dependent from claim 42, and claim 47 is dependent from claim 46, Applicants respectfully submit that the seeds of claims 39, 44 and 47 must also be free of the prior art and thus are allowable subject matter.

As it is believed that all of the rejections set forth in the Official Action have been fully met, favorable reconsideration and allowance are earnestly solicited.

If, however, for any reason the Examiner does not believe that such action can be taken at this time, it is respectfully requested that he/she telephone applicant's attorney at (908) 654-5000 in order to overcome any additional objections which he might have.

If there are any additional charges in connection with this requested amendment, the Examiner is authorized to charge Deposit Account No. 12-1095 therefor.

Dated: October 27, 2009

Respectfully submitted,

By____________________

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APPENDIX

Document A

10th International Rapeseed Congress, Camberra, Australia 1999

CHARACTERISTICS OF DOUBLE LOW WINTER RAPESEED LINES WITH INTRODUCED RESTORER GENE FOR CMS OGURA

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ABSTRACT

The main purpose of the investigations was to obtain the restorer lines for CMS ogura system with low glucosinolate content and good yielding ability. The plants used for the study were selected in F_3 generation of hybrids between CMS lines with low glucosinolate content or double low fertile lines and restorer lines with glucosinolate content of about 60 μM/g of seeds. The 30 selected plants homozygous in respect of restorer gene alleles were characterized by content of total glucosinolates in the range of 10,7 - 19,6 μM/g of seeds. The yield components (number of seeds per pod, 1000 seed weight) revealed high variability. The obtained results showed that in genome of the restorer lines with low glucosinolate content the isozyme marker PGI - 2 and PCR-RAPD molecular marker OPC 02 are not lost with the fragment of DNA responsible for high glucosinolate content.

KEYWORDS: glucosinolates, yield components, molecular markers

INTRODUCTION

The development of hybrid varieties of rapeseed with the use of CMS *ogura* has been one of the main breeding objectives in the past few years. The lack of restorer lines with appropriate qualitative and agronomical traits is the factor limiting the utilization of CMS *ogura* in breeding of restored hybrid varieties. This difficulty is due to the origin of restorer lines in *Brassica napus*. Restorer gene has been introduced to the rapeseed genome from radish (*Raphanus sativus*) genotype (Heyn 1976). Obtained recombinants were characterized by low seed set in pods attributed to a high rate of embryo sac abortion (Pellan - Delourme and Renard 1988; Delourme et al 1991) and strong linkage of restorer alleles with genes determining high glucosinolate content (Delourme et al 1995).

These recombinants retained too much genetic information from radish. Investigations conducted by Delourme et al (1995) revealed that the improvement of these traits is possible by backcrosses with double low lines and elimination of radish genetic information.

The aim of the investigations undertaken by Oil Crop Department of IHAR was to obtain restorer lines with low glucosinolate content and good yielding ability.

MATERIAL AND METHODS

Material for investigations was constituted of:

• restorer line R obtained from INRA - France within the licence agreement, heterozygous in respect of restorer gene alleles, with total glucosinolate content of about 60 μM/g of seeds,

- double low lines of winter rapeseed with glucosinolate content 5,1 - 11,8 μM/g of seeds,
- CMS *ogura* lines with glucosinolate content 12,2 - 16,0 μM/g of seeds.

Investigations were carried out in F_3 generation selected from hybrids between restorer line R and low glucosinolate lines with sterile or normal cytoplasm.

Selection of genotypes with restorer gene alleles was carried out on the phenotypic expression and with the use of isozyme marker PGI-2 (Delourme and Eber 1992). The presence of molecular marker of restorer gene RAPD-OPC02 (Delourme et al 1994) was investigated only in lines characterized by low glucosinolate content. The analyses of glucosinolates were performed with the method of gas chromatography of silyl derivatives of desulfoglucosinolates. Seed set in pods and 1000 seed weight were evaluated in F_3 progeny on ten plants from each of the 44 examined lines.

RESULTS

Segregating population F_2 of about 2500 plants were obtained by crossings of starting restorer line with CMS *ogura* and double low lines with normal cytoplasm. From this population 44 lines were selected. They were characterized by glucosinolate content from 13,6 μM/g of seeds to 25,6 μM/g of seeds and relatively regular meiotic behaviour, but still disturbed by the presence of some univalents and in some cases multivatents. In F_3 progeny obtained from these lines 429 male fertile plants were selected for futher investigations. After examination with the use of PGI-2 marker it was observed that 215 plants were homozygous (Rfo Rfo) and 214 plants were heterozygous (Rfo rfo). The coefficient of variability in respect of glucosinolate content was very high for both groups of plants (Figure 1), but 30 homozygous plants with total glucosinolate content not exceeding 20 μM/g of seeds were selected. The main yield component number of seeds per pod was not dependent on glucosinolate content (Figure 2). The correlation coefficient between these two traits was statistically not significant (r = - 0,117). However, the mean value of seed set per pod for homozygous plants was 15,88 and for heterozygous plants 15,62. It was below the mean value for standard variety which amounted in the same growing conditions 22,4, but several plants not exceeding 15 μM/g of seeds of glucosinolates (Polish norm for sowing material) revealed seed set per pod in range 15 to 24,5.

The presence of PGI-2 and RAPD OPC02 markers was observed in all low glucosinolate homo- and heterozygous genotypes.

CONCLUSION

The selected double low restorer lines with improved productivity will be used to produce restored F_1 hybrids. Two investigated markers PGI-2 and OPC02 closely linked to the restorer gene are not lost in low glucosinolate recombinants together with the fragment of DNA responsible for high glucosinolate content.

Figure 1

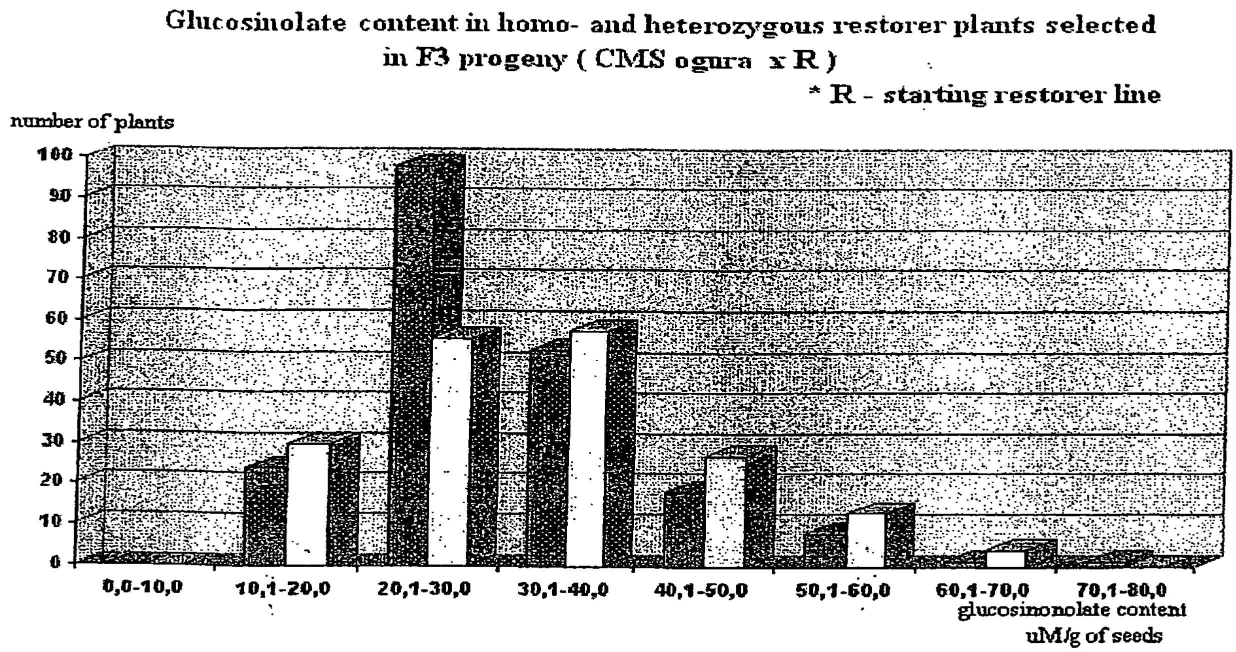

Figure 2

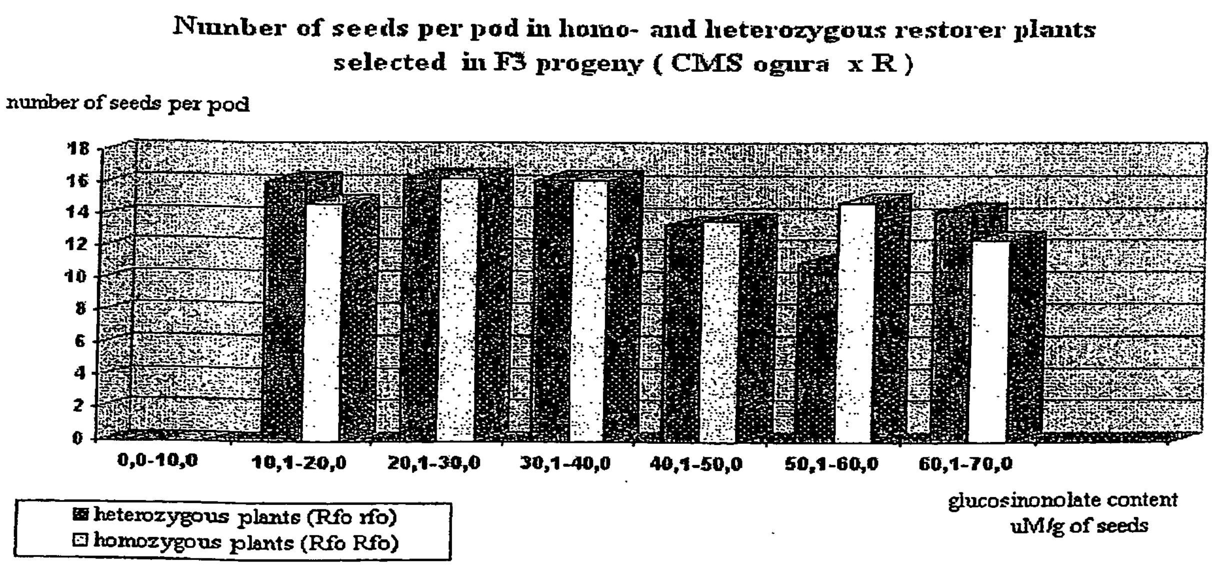

Table 1: Characteristic of homozegous and heterozygous lines with glucosinolate content lower than 20 μM/g of seeds.

	Total glucosinolates (μM/g of seeds)	Aliphalic glucosinolates (μM/g of seeds)	Number of seeds per pod	1000 seed weight	Oil (%)
HOMOZYGOUS LINES					
mean	14.97	11.87	14.87	3.86	40.87
max	19.61	17.11	27.50	5.10	46.90
min	10.29	6.74	6.73	2.70	31.40
coefficient of variability	2.78	2.92	5.48	0.75	3.74
standard deviation	18.56	24.61	36.85	19.57	9.16
HETEROZYGOUS LINES					
mean	16.28	12.97	16.21	3.78	40.65
max	19.93	16.00	33.50	5.60	45.70
min	10.77	7.99	5.40	2.40	33.80
coefficient of variability	2.51	2.55	6.73	0.89	3.63
standard deviation	15.41	19.62	41.52	23.67	8.93

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Document B

10th International Rapeseed Congress, Camberra, Australia 1999

DOUBLE LOW RESTORED F1 HYBRIDS CAN BE PRODUCED WITH THE Ogu-INRA CMS IN RAPESEED.

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ABSTRACT

Breeding double low restorer lines for the Ogu-*INRA Cytoplasmic Male Sterility system in rapeseed (*Brassica napus *L.) has been a major objective during the past few years. After introgressing a restorer gene,* Rfo, *from radish into rapeseed, backcross and pedigree breeding was achieved and resulted in restorer lines with an improved female fertility and regular meiotic behaviour. However, the use of these restorer lines in double low F1 hybrid breeding has been slowed down because of a tight linkage between the radish introgression and high glucosinolate content. Double low restorer lines have now been selected and are being used to produce double low restored F1 hybrids.*

KEYWORDS: Brassica napus, *restorer line, radish introgression*

INTRODUCTION

Breeding restorer lines for the *Ogu*-INRA Cytoplasmic Male Sterility (CMS) system (Pelletier *et al* 1983, 1987) in rapeseed (*Brassica napus* L.) has been a major objective for the last years. A restorer gene *Rfo* was introgressed from radish (*Raphanus sativus* L.) into rapeseed (Heyn 1976) through intergeneric hybridisation. Extensive backcross and pedigree breeding (Delourme *et al* 1991) were necessary to improve the low female fertility of the restorer lines which was attributed to a long radish genetic information remaining around the *Rfo* gene or elsewhere in the genome (Pellan-Delourme and Renard 1988). This breeding resulted in restorer lines (2n=38) of which female fertility was equal to the one of rapeseed maintainer lines and meiotic behaviour was greatly stabilised, thus leading to a more regular transmission of the restorer gene through backcross or self pollination (Delourme *et al* 1995).

Some radish DNA still remains around the introgressed *Rfo* gene. A radish isozyme allele at *Pgi-2* locus was found to be tightly linked to the *Rfo* gene (Delourme and Eber 1992). RAPD and RFLP markers were then identified, the polymorphic DNA fragments being associated either with the restorer allele or with the sterility maintainer allele (Delourme *et al* 1994; 1998). Some lines were identified to have lost the *Pgi-2* allele of radish, which indicated that the introgression can be modified (Delourme and Eber 1992).

The use of the restorer lines in double low F1 hybrid breeding has been slowed down because of a tight linkage between the radish introgression and high glucosinolate content (Delourme *et al* 1995; 1998). However, different low-glucosinolate restorer lines have been selected since 1992. These lines still carry or have lost the radish *Pgi-2* allele.

The origin, the characteristics and the molecular characterisation of the different restorer lines obtained are described in this paper.

MATERIAL AND METHODS

Plant material

The origin of the restored *B. napus* lines carrying the *Rfo* radish restorer gene was previously described (Pellan-Delourme and Renard 1988). In 1989, an improved family ('R20') was selected, giving rise to a progeny with a good female fertility (Delourme *et al*, 1991). Breeding of this material was continued through self pollination and backcrosses with double low lines.

RAPD analyses

The RAPD markers identified in Delourme *et al* (1994; 1998) were tested on the different restorer lines in order to characterise the rearrangements that have occurred in the introgression. The procedure for RAPD analyses was as described in Foisset *et al* (1996).

RESULTS AND DISCUSSION

Breeding double low restorer lines

'R40', an homozygous single low winter rapeseed restorer line was derived from the 'R20' family improved for female fertility (Delourme *et al* 1991) through pedigree breeding (F6) (see Fig 1.). This winter restorer line was supplied to private breeding companies in 1992. Two spring restorer lines were also supplied in 1992 and 1994. They were derived from backcrosses first, with 'Samouraï', a winter double low line and then, with spring double low lines. One of these spring restorer lines had lost the *Pgi-2* allele of radish (Fig 1). Through successive backcrosses with 'Samouraï', some low glucosinolate winter restorer lines were obtained in 1995. These lines still carry or not the *Pgi-2* allele of radish.

As previously stated in Delourme *et al* (1995), the female fertility of the restored plants was not affected by the loss of radish *Pgi-2* allele when they are at the heterozygous stage but the homozygous restored plants which have lost *Pgi-2* allele of radish showed a very poor seed set. This was explained by the fact that these plants lack a rapeseed chromosomal segment (they have not recovered the rapeseed *Pgi-2* allele).

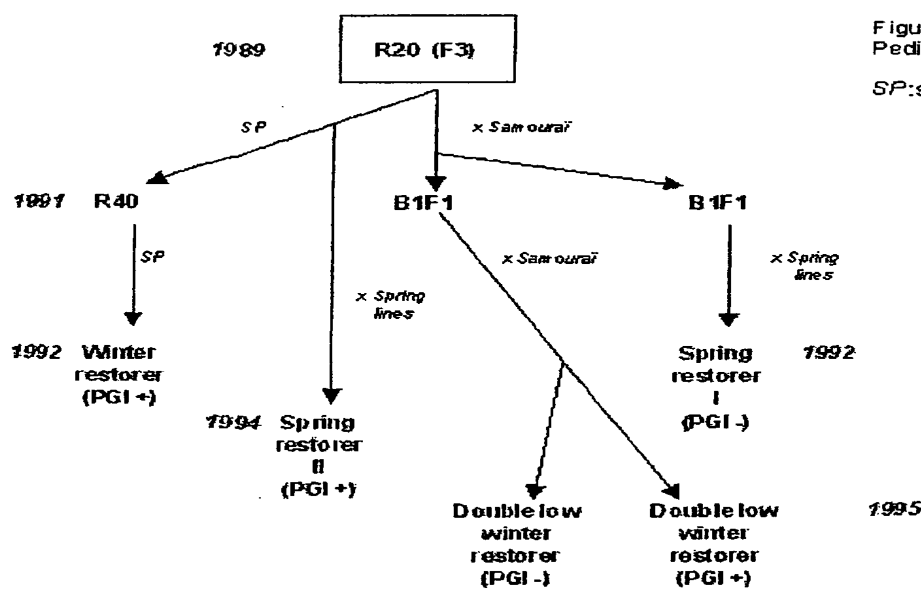

Figure 1:
Pedigree of the restorer lines

SP: self pollination

Characterisation of the radish introgression in different restorer lines

RAPD markers carried by the radish introgression were tested on several restorer lines. Table 1 summarises the different types observed. It shows that some markers were lost in PGI - or/and in low glucosinolate restorer lines. RAPD6 and RAPD14 were lost in low glucosinolate restorer lines, independently of the presence or absence of *Pgi-2* allele of radish, indicating that the elimination of radish *Pgi-2* allele is not necessary to get low glucosinolate restorer lines.

Table 1: Presence/absence of RAPD markers of the radish introgression in different types of restorer lines.

	PGI +			PGI -	
GLS [a]	high	intermediate	low	high	low
D02.1000	+	+	+	-	-
Pgi-2	+	+	+	-	-
RAPD7b	+	+	+	-	-
RAPD13b	+	+	+	+	+ or -
C02.1050	+	+	+	+	+
~~RAPD1~~	~~+~~	~~+~~	+	+	+
RAPD2	+	+	+	+	+
RAPD4	+	+	+	+	+
RAPD7a	+	+	+	+	+
RAPD8	+	+	+	+	+
RAPD9	+	+	+	+	+
RAPD21	+	+	+	+	+
RAPD22	+	+	+	+	+
RAPD6	+	+	-	+	-
RAPD14	+	+	-	+	-

(a) GLS content was assessed through HPLC; High, intermediate and low GLS content correspond to more than 35, between 25 and 35 and less than 20 µmoles/g seed, respectively.

Breeding double low restored F1 hybrids

A double low winter restorer line was used to produce restored F1 hybrids. These F1 hybrids were tested in field trials and assessed for glucosinolate content. It showed that we are able to obtain winter restored F1 hybrids with a glucosinolate content ranging from 9 to 18 µmoles/g seed depending on the female parent and on the year. This line is being used in backcross breeding in order to diversify the winter and spring restorer lines.

To develop early sowing, to improve resistance to lodging and resistance to frost as well as to simplify harvesting, INRA, in collaboration with SERASEM, is breeding semidwarf F1 hybrids. Thus a semidwarf restored F1 hybrid (B017) was produced with the double low

winter restorer line and is now in second year of official trials for registration in France and Great Britain.

CONCLUSION

Our results indicate that it is possible to get double low restored lines through conventional breeding and to use these lines in double low F1 hybrid breeding. However, the radish introgression is still large in these lines. It still needs to be reduced in order to decrease the probability of rearrangements which could lead to material with a poor agronomic value. Thus, programs aiming at cloning the *Rfo* gene or at reducing the size of the introgression are in progress.

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